Protein microcrystal manipulation with optical tweezers

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Introduction

X-ray crystallography is the most successful experimental technique to deduce structural information on an atomic level from macromolecules such as proteins and DNA. Most of the steps for protein production, purification and crystallization have been automated during the last years. The same is true for the X-ray diffraction experiments at synchrotron radiation sources, including subsequent data analysis, and in many case only limited user interaction is necessary. However, one important step, the transfer of a crystal from the growth medium to a sample holder is still purely manual. For typical crystals at third generation synchrotrons with dimensions in the range of tens of micrometers this mounting can be performed under stereomicroscopes. Nylon loops or kapton meshes are used to scoop the crystals out of the droplet. However, these manual procedures become difficult for samples with dimensions smaller than 20 µm. Latest synchrotron beamlines, like the microfocus macromolecular crystallography beamline I24 at Diamond Light Source, provide X-ray beams smaller than 10 µm on a routine basis. These significantly smaller beam sizes open the technique to crystals down to 1 µm. We have used laser tweezers to overcome the manipulation problems with such small samples. In a series of experiments we could demonstrate the applicability of optical tweezers to manipulate microcrystals from two different proteins, human insulin from the commercial microcrystalline drug preparation Ultralente [1] and polyhedra (PH) microcrystals from a cytoplasmatic polyhedrosis virus [2]. The pharmaceutical effect of Ultralente crystals depends on their size. Therefore the crystallization conditions have been optimized to keep it constant. They are all of regular rhombohedral shape with 25 × 25 × 5 µm dimensions. The size of PH crystals, however, being from natural sources, differs significantly. Cypovirus polyhedra are typically cubes with 5-7 µm edges. Crystals larger than 10 microns in diameter exist, but constitute less than about 1/10,000 of the population. For the X-ray diffraction experiment it is of highest importance to be able to find and mount the biggest crystal. Both crystal types are relatively stable and were readily available in large quantities for these tests.

Experimental

The laser tweezers setup at CLF was used throughout these experiments [3,4]. To find the most suitable laser source we used both a green 514.5 nm Ar ion laser and a Nd:YAG near infrared laser (1064 nm) coupled into an inverted microscope using an objective lens with high numerical aperture (N.A. 1.2). Multi-trap configurations were produced by an acousto-optic deflector in the beam path.

PH crystals were sorted in glass capillaries, while for the mounting experiments the micromesh crystal holders were directly placed in the droplet containing the microcrystals and supported by a small holder designed at CLF. Micromeshes [5] with 10 µm holes were used for the PH and 25 µm holes for the Ultralente sample mounting experiments.

Results and discussion

The Ar-ion laser at a laser power of less than 50 mW was observed to cause instant radiation damage to the sample holders and was therefore not considered for further experiments. Sample holders lasted for several minutes in the 1064 nm laser before they showed damage.

Crystal manipulation turned out to be straightforward for both sample types. The crystals of both geometries trapped easily and could be moved in three dimensions. Crystals could be lifted and moved above other crystals lying on the bottom of the droplet. Figure 1 shows a typical distribution of PH crystals before and after sorting the biggest crystals in the capillary. We did not observe visible laser damage to the crystals using the 1064 nm laser wavelength.

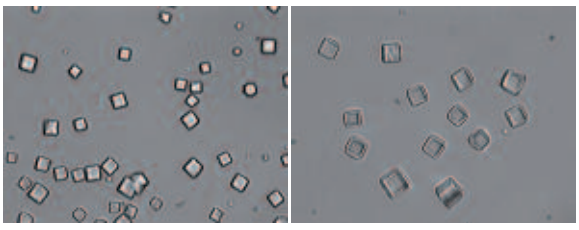

Figure 1. Typical PH crystal sample with average crystal dimensions of 5 – 7 µm (left). 8 – 10 µm crystals after sorting (right).

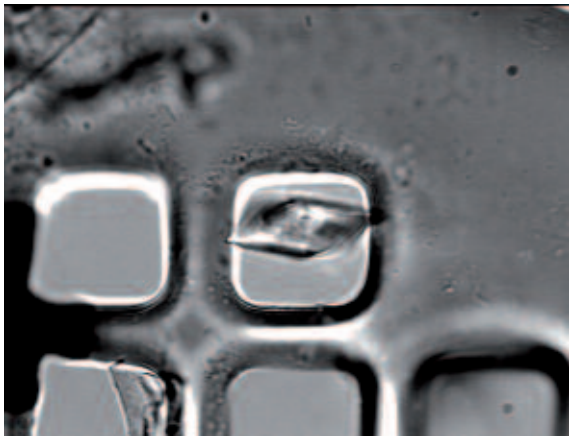

Figure 2. Ultralente microcrystal optically mounted on micromesh.

For crystal mounting we faced two issues. Crossing the interface of the sample holder changes the focal properties of the optical trap and therefore the process needs to be very slow and at least 50 µm above the holder surface. The second problem was that in the case of the crystals being smaller than the holes in the meshes we were not able to attach them to the holder and the crystals fell through the holes once the optical trap was switched off. Nevertheless it was possible to place several Ultralente crystals on a micromesh (figure 2).

Conclusions

Laser tweezers provide a unique tool to easily sort and mount protein microcrystals in solution. The current limitations are the radiation damage to currently available sample holders and a re-design of the micromesh is needed to facilitate optical loading. The next step of our ongoing research is to investigate possible laser damage to protein microcrystals by this novel manipulation technique using X-ray diffraction.

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